Physiological and biochemical traits of dormancy release and growth resumption in Japanese cedar in the warm-temperate zone

Forest Science

Online Resource 2

Measurement of starch contents

For each tree, after the shoots were milled and sieved through a 212-μm mesh into fine powder, 0.10 g of fine powder was weighed into a 15-mL centrifuge tube (VIO-15BN; AS ONE, Osaka, Japan). Then, 5 mL of 80% (v/v) ethanol were added to the tube and the mixture incubated for 5 min at 80°C. Next, 5 mL of 80% (v/v) ethanol were added to the tube, followed by centrifugation for 10 min at 1870 × *g*, after which the supernatant was discarded. The precipitate was resuspended in 10 mL of 80% (v/v) ethanol, centrifuged as described above, and the supernatant was poured off. Then, 3 mL of a solution consisting of thermostable α-amylase (8300 units mL^{-1} on soluble starch) and sodium acetate buffer (100 mmol L^{-1}, pH 5.0) mixed in a ratio of 1:30 (v/v) were added to the precipitate and the mixture incubated for 6 min at 100°C. Next, 0.1 mL of amyloglucosidase (20 units) was added to the precipitate, followed by incubation for 30 min at 50°C. The suspension was transferred to a volumetric flask and diluted to 50 mL with deionized water to generate sample solution. An aliquot of each sample solution was centrifuged for 10 min at 2350 × *g*, and 0.25 mL of the centrifuged sample solution was transferred into a 1-cm wide glass cuvette. Then, 1.8 mL of deionized water, 0.1 mL of buffer solution (pH of 7.6), and 0.1 mL of a mixture of $NADP^+$ and ATP were added to each cuvette, and the absorbance at 340 nm (S1) was measured using a spectrometer (U-5100; Hitachi, Ltd., Tokyo, Japan). Subsequently, a 0.02-mL solution containing hexokinase and glucose-6-phosphate dehydrogenase was added to each cuvette, and the absorbance at 340 nm (S2) was measured. A blank sample solution consisting only of deionized water was prepared, and S1 and S2 were measured in the same way (S1 and S2 for the blank are described as B1 and B2, respectively). Based on these measurement values, the amount of starch (mg) in each sample was calculated by multiplying the starch concentration (g L^{-1}, equal to mg mL^{-1}) (formula 2) by its adjusted volume, i.e., 50 mL. Finally, the content of starch on a dry weight basis (mg g^{-1}) was calculated by dividing the amount of starch by the dry weight of the sample.

[Formula 2] Starch concentration (g L^{-1}) = Solution volume in the cuvette (mL) × Molecular weight of d-glucose (g mol^{-1}) × 0.9 × {(S2 – S1) − (B2 − B1)}/Extinction

coefficient of NADPH at 340 nm (L mol^{-1} cm^{-1})/Light path (cm)/Volume of sample solution added to the cuvette (mL)